Acta Societatis Botanicorum Poloniae

DOI: 10.5586/asbp.3618

Publication history
Received: 2018-04-03
Accepted: 2019-02-12
Published: 2019-04-02

Handling editor
Krzysztof Spalik, Faculty of Biology, Biological and Chemical Research Centre, University of Warsaw, Poland

Authors' contributions
JU and PK contributed to the design of the research, all of the experimental processes, conducted the experiments, analyzed the data, and wrote, submitted, and revised the manuscript; BK contributed to experimental process

Funding
The project was financed under the program of the Polish Minister of Science and Higher Education "Strategy of Excellence – University of Research" in 2018–2019; project number 0019/SDU/2018/18.

Competing interests
No competing interests have been declared.

Citation
Urbaniak J, Kwiatkowski P, Kozak B. Genetic differentiation of *Allium sibiricum* L. populations in Poland based on their morphological and molecular markers. Acta Soc Bot Pol. 2019;88(1):3618. https://doi.org/10.5586/asbp.3618

ORIGINAL RESEARCH PAPER

Genetic differentiation of *Allium sibiricum* L. populations in Poland based on their morphological and molecular markers

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Abstract
Allium sibiricum is one of the rarest plant species in the Sudetes and Carpathians. Inter simple sequence repeat DNA (ISSR) and morphological analyses were conducted to study the biogeographical relationships between geographically disjunctive populations of *A. sibiricum* in the Carpathians and Sudetes. The results clearly differentiated the Carpathian and Sudetes populations, but also showed a relatively high level of genetic similarity in specimens within certain groups of the Sudetes and Carpathian populations. The plants collected in the Karkonosze probably belong to a mountain form of *A. schoenoprasum* morphotype C which inhabits natural sites there. In contrast, the study found morphologically and genetically different plants inhabiting Pilsko Mt in the Carpathians. The plants from the Carpathians were present in scattered sites probably due to the activity of Vlach shepherds and the formation of large pastures. The species enlarged its local range due to this type of anthropopressure and likely hybridized with the cultivated *Allium* plants introduced by the shepherds. This may indicate that the populations on Pilsko Mt are of a partly anthropogenic character.

Keywords
chives; Alliaceae; *Allium*; morphotypes; phylogeography; ISSR; Poland

Introduction

The genus *Allium* L. appears to be one of the largest in monocots whose taxonomic classification is still problematic. Fritsch et al. [1] determined that the genus *Allium* comprised more than 800 species widely distributed across the Holarctic region and to date, no comprehensive monograph of the genus has been written. This is probably due to its complicated taxonomy, which has a proliferation of synonyms as well as a general disagreement as to the taxonomic rank in the genus [2]. In the case of the genus *Allium*, not only is its taxonomy unclear, but its biogeography is as well. It is difficult to ascertain when and how the *Allium* representatives reached Europe, though most probably from their Asian diversity center, thereby inhabiting new territory and new sites. The migration of plants in this case is a key factor responsible for the observed intraspecific diversity [3,4]. Plants inhabiting new locations and adapting to specific sites often develop local morphologically and/or genetically diverse ecotypes and this seems to be the main reason why many similar populations of rare species were also recognized as having less genetic diversity than more widely distributed species [5].

Allium sibiricum is diploid and one of the most important species of the section *Schoenoprasum*. Similarly to the other representatives from the section, it is widespread in Eurasia and North America and comprises numerous morphological wild variants and cultivars, which form a complex of local, polymorphic populations composed of different plant morphotypes [6,7]. The geographically distinct populations composed of different plant morphotypes can differ in shoot morphology, as well as in the appearance of inflorescences, their ecological requirements, geographical distribution, and can be considered to be a separate taxonomic rank [6,8–10]. This was probably the reason why *A. schoenoprasum* L. was divided into four morphotypes depending on their habitat and distribution according to their occurrence at various sea levels [10,11]. According to Duchoslav et al. [10], in Central Europe there are two commonly found morphotypes of *A. schoenoprasum*. Morphotype A [*A. schoenoprasum* subsp. *riparium* (Opiz) Čelak. which exists in populations situated along river valleys] and morphotype C [*A. sibiricum* L., which occurs in mountain localities and includes robust plants (up to 50 cm in height) which can be found in the Eurasian mountain ranges]. In the case of morphotype C, three synonyms are used in scientific literature: (***i***) *A. schoenoprasum* subsp. *sibiricum* (L.) Hayek & Markgraf, (***ii***) *A. schoenoprasum* subsp. *alpinum* (DC.) Čelak, and (***iii***) *A. schoenoprasum* var. *alpinum* DC. in Lam. [10]. This morphotype has been found in the Sudetes (Hrubý Jeseník Mts and Lužické Hory Mts) in the Czech Republic and in the Polish and Czech Karkonosze Mts [12]. Two populations similar to morphotype C can also be found on Hala Miziowa and Hala Cebulowa (Pilsko Massif, Carpathians) in the Beskidy Mts and are widely known in Poland as the Carpathian populations of *A. sibiricum* [13–16] or *A. schoenoprasum* L. subsp. *sibiricum* (L.) Hartm. [17].

In this paper, we present the results of research on the genetic structure and diversity of the populations of rare and endangered *A. sibiricum* from several disjunctive geographical regional locations in Poland. Molecular ISSR markers were used to determine the genetic relationships between different populations of *A. sibiricum*. A statistical analysis of their morphological characters was performed in order to determine the relationships between the populations which inhabit geographically separate regions – the Beskidy and Karkonosze Mts.

Material and methods

Study regions and sampling of the populations

Allium sibiricum L. (*A. schoenoprasum* L. morphotype C) belongs to the section *Schoenoprasum* Dumort., and is one of the most widely dispersed species across all of Europe, Asia, and North America [11]. For the study, samples collected from seven populations of species across the geographical range in Poland were used (Tab. 1, Fig. 1). Molecular analysis was conducted on plants from all populations. In the case of morphological analysis, plants from populations located at Kocioł Małego Stawu were not studied. Specimens were randomly sampled in populations at distances which generally depended on the spatial extent of the populations (2–3 m). Fresh leaves (one–two) were collected in plastic zip bags, dried in silica gel, transported to the laboratory, and stored until the DNA extraction. All of the plant material was collected in one vegetative season between June and July 2016. Morphological characteristics of *A. sibiricum* specimens were measured in situ: stem height, stem diameter in the middle, stem diameter below the umbel, umbel height and width, sepal height and width, and pedicel length (Tab. 2).

DNA extraction and molecular analysis

The genomic DNA was isolated from disrupted cells with a Mixer Mill MM400 (Retsch, Germany) using a DNeasy Plant Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. The quality and quantity of the DNA were determined using 1% TBE electrophoresis. The inter simple sequence repeat (ISSR) microsatellite markers were selected to study the genetic diversity of the populations of *A. sibiricum*. The ISSR

Tab. 1 The origin of the studied populations of *Allium sibiricum* L. in Poland.

Population name	Origin of plants	Altitude (m a.s.l.)
	Karkonosze Mts (Sudetes)	
KWS	Kocioł Wielkiego Stawu, northern slope	1,260
KMS*	Kocioł Małego Stawu, northern slope	1,159
KO	Kopa Mt, northeastern slope	1,278
KL	Kocioł Łomniczki, northern slope, along Łomniczka stream	1,217
JG	Living Gene Bank (Jagniątków)	625
	Beskidy Mts (Carpathians)	
HC	Hala Cebulowa, peat bog	1,301
HM	Hala Miziowa, peat bog	1,282

* Plants from Kocioł Małego Stawu were used only in molecular analysis.

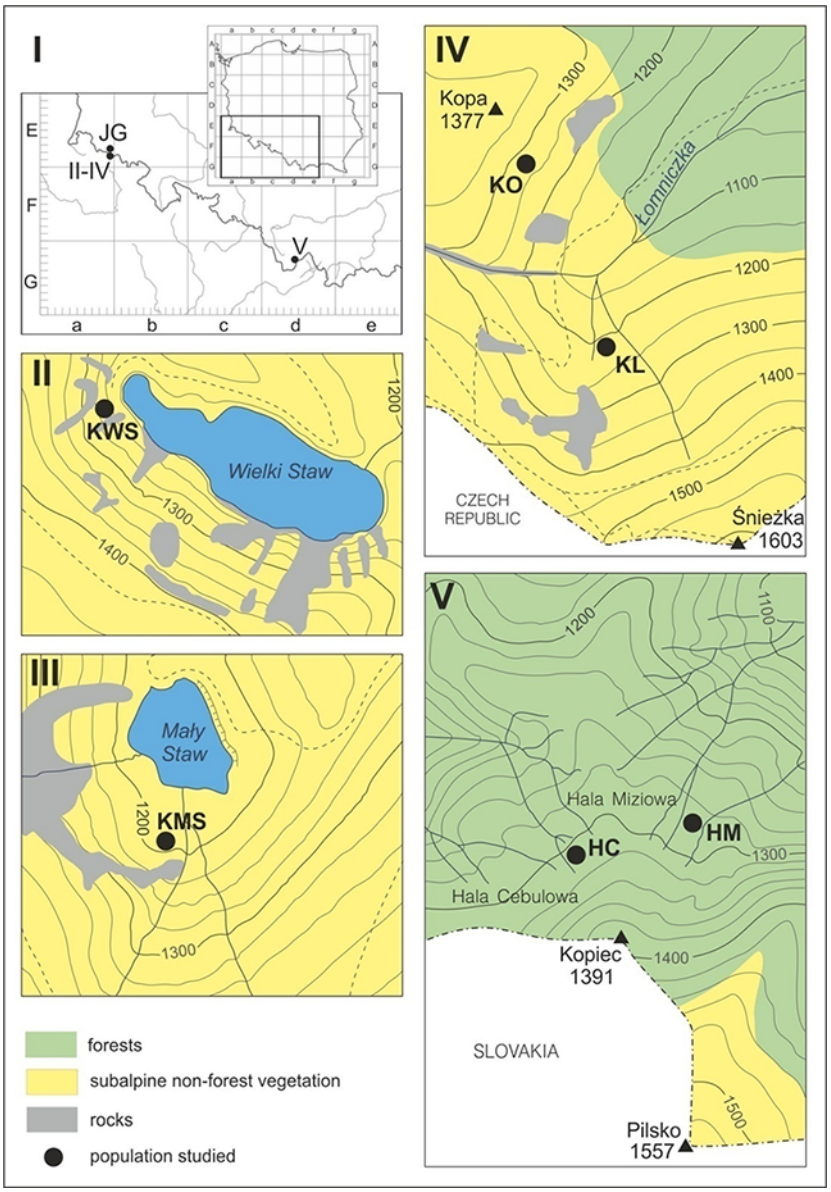

Fig. 1 Location of the studied populations of *Allium sibiricum* L. in Poland. Population abbreviations are the same as in Tab. 1.

Tab. 2 Descriptive statistics of morphological characters in studied populations of *Allium sibiricum* L.*

Morphological character	Studied population of *A. sibiricum* (min–max, mean ±*SD*)						
	Kocioł Wielkiego Stawu	Kopa Mt	Kocioł Łomniczki	Living Gene Bank	Hala Miziowa	Hala Cebulowa	*F* and *p* values
Number of individuals (*n*)	12	27	28	24	31	28	-
Stem height (cm)	31.0–55 48.3 ±9.58 ^{a,c,e,f}	25.0–47 36.7 ±5.71 ^{b,c,e,f}	26.5–51 42.7 ±5.56 ^{a,b,c,d,e,f}	26.0–58.5 42.7 ±8.22 ^{c,d,e,f}	45.0–77 59.1 ±7.51 ^{a,b,c,d,e}	49.0–74 58.0 ±6.03 ^{a,b,c,d,f}	75.6; <0.05
Stem diameter in the middle (mm)	2.4–3.3 2.9 ±0.37 ^{a,c,e,f}	1.7–3.8 2.6 ±0.45 ^{b,e,f}	1.7–3.7 2.9 ±0.52 ^{a,c,e,f}	2.1–4.2 2.9 ±0.53 ^{d,e,f}	2.8–5.5 3.8 ±0.75 ^{a,b,c,d,e}	2.9–4.9 3.9 ±0.52 ^{a,b,c,d,f}	54.9; <0.05
Stem diameter below the umbel (mm)	1.8–4.5 2.5 ±0.98 ^{a,b,c,f}	1.3–2.3 1.7 ±0.26 ^{a,b,c,d,e,f}	1.5–2.8 1.9 ±0.33 ^{a,b,c,d,e,f}	1.7–3.3 2.3 ±0.43 ^{b,c,d}	1.5–3.6 2.4 ±0.44 ^{b,c,e}	2.0–3.9 2.6 ±0.43 ^{a,b,c,f}	9.7; <0.05
Umbel height (mm)	22.0–30.4 25.8 ±2.86 ^{a,b,c,e,f}	17.0–29 21.9 ±2.17 ^{a,b,d,e,f}	17.0–27.4 22.1 ±2.64 ^{a,c,d,e,f}	19.8–29 24.0 ±2.65 ^{b,c,d,e}	22.7–35.5 28.6 ±3.36 ^{a,b,c,d,e}	23.3–34.7 28.3 ±2.91 ^{a,b,c,f}	46.3; <0.05
Umbel width (mm)	28.9–43 36.7 ±4.66 ^{a,b,e,f}	22.8–37.8 31.2 ±3.82 ^{a,b,d,e,f}	23.0–38 29.8 ±4.46 ^{b,c,d,e,f}	29.0–45.5 35.3 ±4.19 ^{b,c,d,e,f}	35.0–50.1 42.1 ±3.28 ^{a,b,c,d,e}	33.9–47.6 42.3 ±3.38 ^{a,b,c,d,f}	44.8; <0.05
Tepals lenght (mm)	9.6–12.1 11.1 ±0.82 ^{a,b,c,d,f}	8.6–12.8 10.8 ±0.89 ^{a,e,f}	10.0–14.7 12.1 ±1.27 ^{a,c,e,f}	9.4–12.1 11.3 ±0.63 ^{a,d,e,f}	9.7–14.3 12.3 ±1.24 ^{b,c,d,e}	11.0–14.5 12.4 ±0.91 ^{a,b,c,d,f}	12.5; <0.05
Tepals width (mm)	1.7–2.8 2.1 ±0.45 ^{a,b,c,f}	1.8–3.2 2.3 ±0.27 ^{a,b,d,e,f}	1.8–3 2.2 ±0.3 ^{a,c,d,e,f}	1.8–3.5 2.6 ±0.41 ^{b,c,d}	2.3–3.6 2.7 ±0.22 ^{a,b,c,e}	2.2–3.9 2.8 ±0.29 ^{a,b,c,f}	19.9; <0.05
Pedicel length (mm)	5.4–8.8 7.7 ±1.2 ^{a,b,c,e,f}	4.0–9 6.1 ±1.28 ^{a,b,d,e,f}	4.5–6.9 5.6 ±0.61 ^{c,d,e,f}	5.6–12 7.8 ±1.82 ^{b,c,d,e}	4.9–9.6 7.1 ±1.19 ^{a,b,c,d,e}	5.3–9 7.0 ±1.09 ^{a,b,c,f}	12.9; <0.05

* Populations that significantly differed (post hoc LSD test, $p < 0.05$) are marked by the same letter.

markers are commonly known to be highly polymorphic and are useful in studies on genetic diversity and species relationship, enabling closely related specimens to be easily differentiated [7,18,19]. ISSR has also been successfully employed to assess hybridization and to detect hybrid taxa [20–23]. Many studies have demonstrated the efficiency of these markers in both phylogenetic analyses and in determining the taxonomic relationships of *Allium* species as well as in population and cultivar genetics studies [7,24,25]. Prior to the study, more than 50 ISSR primers were checked for their usefulness in determining population differentiation in the genus *Allium* based on our own experience or the experience of other authors [26,27]. A total of 10 primers (ISSR: 807, 810, 811, 815, 834, 835, 840, 841, 855, 857, 861) showed a satisfactory amplification and generated an acceptable number of polymorphic bands. Any primers showing scattered polymorphism or generating monomorphic bands were excluded from further studies. The number of amplified products varied from four to nine within a size range of 100 to 1,000 bp, depending on the specific ISSR primer. PCR reactions were performed in a 20-μL reaction tubes with Dream Taq reaction buffer containing $MgCl_2$, 0.2 mM dNTP mix, 1 U DreamTaq DNA polymerase (Thermo Fisher Scientific, USA), 0.5 mM ISSR primer, and 1 μL of total genomic DNA. The PCR cycle consisted of an initial denaturation at 95°C for 6 min followed by 33 cycles at 95°C for 30 s, the adequate annealing temperature was tested using the gradient method for 30 s, a 72°C elongation for 30 s, with a final extension of 10 min at 72°C. A Veriti Thermal Cycler (Life Technologies, USA) was used. The PCR ISSR amplification products were separated in 1% agarose gel, visualized with Simply Safe stain (Eurx, Poland), and photographed. All molecular analyses were performed at the Department of Botany and Plant Ecology at the Wrocław University of Environmental and Life Sciences.

Molecular data analysis

To determine the relationship between the *A. sibiricum* populations, the results were run on agarose gel and were compared with the DNA mass ruler (Thermo Fisher Scientific, USA), photographed, and analyzed using CLIQS [28] software. The markers were encoded as 1 (present) or 0 (absent) in a binary matrix and used for further calculations. The analyses of the molecular variance (AMOVA) within and between the populations or between the groups (F_{ST}) were performed using ARLEQUIN 3.5.1 [29] with 1,000 permutations in order to test the partitioning of genetic variation within and among the populations as well as the importance of the main groups of populations. Nei's genetic identity 3 [30] index was calculated using POPGENE v. 1.32 [31]. Bayesian clustering was applied using STRUCTURE 2.3.4 [32,33] based on an admixture model. The numbers of K from 2 to 7 were tested with 10 replications per K. One million Markov chain Monte Carlo repetitions were applied with burn in period of 2,000,000. The L/K or ln $P(D)$ can be used as an indication of the most likely number of groups, and it usually plateaus or increases slightly after the correct K is reached [34]. Therefore, the height of the modal value of the K or mean $(|L(K)|)/sd(L(K))$ distribution was calculated in order to detect the true K [34] using the structure sum to control the stability of the results [35]. Output data with multiple values of K and hundreds of iterations were analyzed using STRUCTURE HARVESTER [36] with CLUMPP [37]. DISTRUCT [38] and GSVIEW ver. 4.8 [39] were used to produce graphical displays of the STRUCTURE results and computing useful statistics. Unrooted neighbor-joining tree of an interpopulation relationship was analyzed based on the genetic distance [40] of using FAMD 1.3 [41] and SPLITSTREE ver. 4 [42] with 1,000 replicates.

Morphological data analysis

The morphological characteristics of the studied plants were described statistically and analyzed after normality distribution confirmation (Kołomogorov–Smirnov and Shapiro–Wilk tests). The tests showed a normal distribution in the case of all morphological features except for the pedicel length, but in the case of this property, the logarithmic transformation was applied to obtain the normal distribution. In order to evaluate the presence of significant differences between all plant populations, the ANOVA test was

performed. We also performed post hoc test of least significant difference (LSD) to verify the differences in characteristics between the studied populations. Additionally, Student *t* tests were conducted to compare morphological data from two geographically distinct groups of populations, Karkonosze and Beskidy. In all statistical tests, a $p < 0.05$ level was considered to be statistically significant. We have also used principal component analysis (PCA) to verify the differentiation of individual plants from particular populations and to check which morphological characters are responsible for this differentiation. All of the statistical analyses were conducted using STATISTICA 13.3 [43].

Results

Analyses of morphological data

The results of morphological characteristics of *A. sibiricum*, their descriptive statistics, results of ANOVA and post hoc LSD tests are presented in Tab. 2. All plant populations differed significantly in all characteristics. In general, plants from the Karkonosze were smaller than those from the Beskidy (Hala Miziowa and Hala Cebulowa). Plants growing in the Beskidy had significantly higher stems, up to 74 (70) cm high than those growing in the Karkonosze (Student *t* test; $p < 0.05$). As to other characteristics, plants measured in the Beskidy had significantly greater diameter of the stem measured in the middle, up to 5.5 mm (H. Miziowa), umbel higher (34.7–35.5 mm) and wider (47.6–50.1 mm), and greater tepals width (3.8–3.9 mm) than plants from the Karkonosze (Student *t* test; $p < 0.05$). Plants collected from both populations in the Beskidy also had significantly greater stem diameter below the umbel (3.6–3.9) and higher tepals (14.3–14.5) (Student *t* test; $p < 0.05$). In both cases, plants measured respectively in the Kocioł Wielkiego Stawu and Kocioł Łomniczki were noted as higher, but in general means were lower than plants measured in the Beskidy. These results were also corroborated by the results of post hoc LSD test presented in Tab. 2, showing that in almost all characteristics, plants from the Karkonosze differed significantly from plants collected in the Beskidy (LSD test; $p < 0.05$). Four populations from the Karkonosze appeared to be mountain plant populations in the typical morphotype C such as *A. sibiricum* according to [11], and, as it was described in detail above, were in general morphologically different from the Beskidy plants (Student *t* test, $p < 0.05$). The study also statistically compared the differences between plants collected from populations located in Living Gene Bank vs. plants collected in the Karkonosze and separately collected in the Beskidy, finding statistically important differences (Student *t* test, $p < 0.05$). The differentiation between the Karkonosze and Beskidy mountain populations was also confirmed using PCA (Fig. 2, Fig. 3). PCA showed which morphological properties are responsible for the differentiation of plants in populations, and based on the result analysis the first factor explained 55.6% and the second 12.4% of the total variation. The strongest correlation was found between the first factor and five from eight used variables: umbel width (0.891), stem diameter in the middle (0.879), umbel height (0.863), stem height (0.855), and stem diameter in the middle (0.765). Other correlations of variables and the first factor were: tepals width (0.606), tepals height (0.548), pedicel length (0.383). Additional analysis, the scatter plot from the first two components of PCA, showed differentiation of studied plant specimens. Samples from the Beskidy are generally located on the right side, presenting differentiation from samples from the Karkonosze. Although this suggests the existence of two different morphotypes of *A. sibiricum* in both mountain ranges, some transitional forms between specimens were also found, which is also visible in Fig. 2.

Analyses of molecular data

Molecular analysis appeared to confirm the statistical analysis of morphological features. There were differences between plants from the Living Gene Bank and from the other studied populations from the Karkonosze Mts. However, it should be noted that plants from the Living Gene Bank do not belong to natural population, and probably come

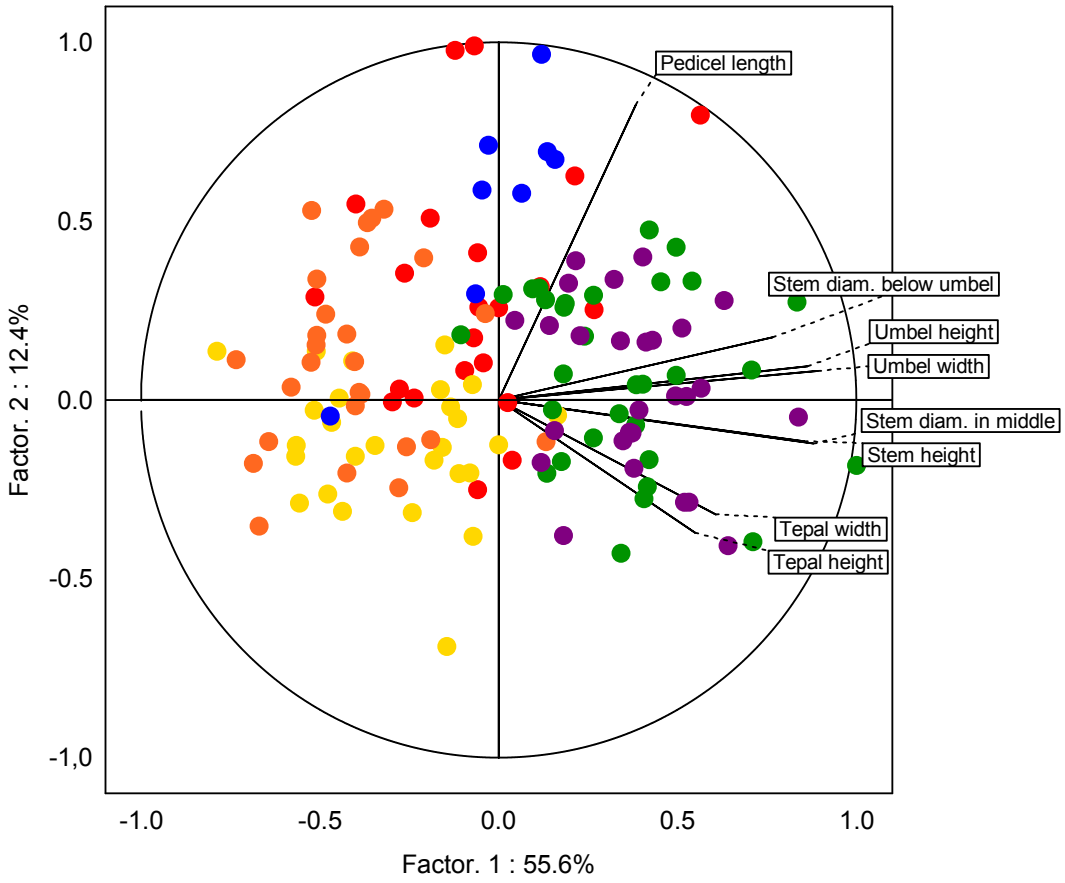

Fig. 2 Biplot graph showing a projection of the morphological characters (variables) on to first two principal factors. Individuals from studied populations of *Allium sibiricum* L. are marked by different colors. Blue – Kocioł Wielkiego Stawu (KWS); orange – Kopa (KO); yellow – Kocioł Łomniczki (KL); red – Living Gene Bank (JG); violet – Hala Cebulowa (HC); green – Hala Miziowa (HM).

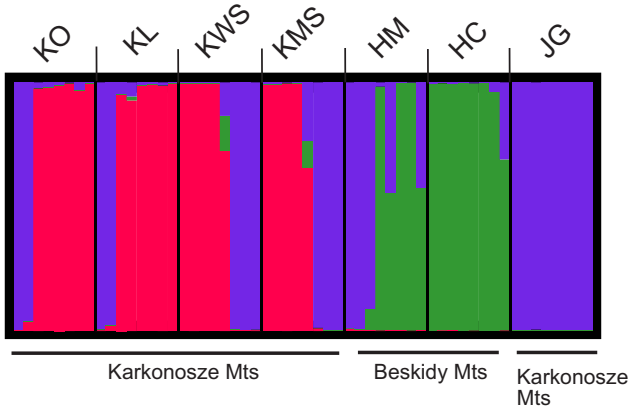

Fig. 3 Results of the Bayesian admixture analysis data for populations of *Allium sibiricum* L. using STRUCTURE software, $K = 3$ (see text for details). Populations are separated by bold vertical lines and each individual is represented by thin line. Abbreviations are the same as in Tab. 1.

from unknown wild origin. The analysis of ISSR results showed a distinction of numerous polymorphic loci that enabled a reliable analysis of molecular variance to be performed. The results, which were based on the AMOVA analysis, showed a high and significant degree of genetic differentiation among the populations of *A. sibiricum* (Tab. 4). A high degree of interpopulation variation (93%) was also found among them, whereas only a 7% variation was found within the populations ($F_{ST} = 0.39$, $p < 0.001$). A large amount of genetic variation was also found among *A. sibiricum* plants from the Karkonosze and Beskidy Mts. Variations were found in the range of 87–94% ($F_{ST} = 0.33–0.37$, $p < 0.001$). Nei's genetic identity values indicated that almost all Karkonosze populations were genetically distant from the populations from the Beskidy Mts. The plants from the Living Gene Bank differed significantly from the rest of the populations from the Karkonosze, which was evidenced by the genetic differences of the *A. sibiricum* populations from the plants in the Living Gene Bank (Tab. 3, Tab. 4).

The STRUCTURE analysis runs for $K = 2–4$ and the analysis of the STRUCTURE HARVESTER showed the highest ΔK for three distinct genetic groups, $K = 3$ (Fig. 3). The composition of the studied genetic group seems to be clearly correlated with the results of morphological analysis. Although specimens from the Karkonosze populations (KWS, KMS, KO, KL, JG) formed a genetically separate group of plants from the other populations, the gene pool was not fully homogeneous. *Allium sibiricum* from

Tab. 3 Analyses of molecular variance (AMOVA) based on ISSR markers for populations *Allium sibiricum* L.

Variation	*df*	Variance components	Percentage of variation	Fixation index F_{ST}	*p*
All studied populations					
Among populations	6	10.3	93		
Within populations	49	7.4	7		
Total	111	15.4	1	0.39	0.001
Karkonosze Mts vs. Beskidy Mts					
Among geographical groups	1	16.4			
Among populations	4	10.2	6		
Within populations	42	7.5	0	0.33	0.001
Karkonosze vs. Living Gene Bank population (Karkonosze Mts)					
Among geographical groups	1	71.6			
Among populations	3	43.8	6		
Within populations	35	5.7	1	0.37	0.001
Beskidy Mts vs. Living Gene Bank population (Karkonosze Mts)					
Among geographical groups	1	19.5			
Among populations	1	27.9	13		
Within populations	21	9.9	0	0.36	0.001

Tab. 4 Parwise Nei's genetic identity [30] for populations of *Allium sibiricum* L. from Poland based on ISSR analysis. Abbreviations of population names as in Tab. 1.

	KO	KL	KWS	KMS	HM	HC	JG
KO	-						
KL	0.513	-					
KWS	0.301	0.264	-				
KMS	0.201	0.192	0.758	-			
HM	0.094	0.128	0.085	0.080	-		
HC	0.068	0.029	0.107	0.110	0.336	-	
JG	0.025	0.018	0.018	0.038	0.007	0.028	-

the Beskidy seemed to confirm the existence of populations represented by genetically distinct specimens, which was also reflected in the plant morphology. The biogeographical differentiation found between the Karkonosze and Beskidy also clearly revealed two distinct groups, which was confirmed by a neighbor net analysis (Fig. 4). The analysis divided all *A. sibiricum* specimens into three groups: the plants from the Beskidy, the group of populations from the Karkonosze and an additional branch representing the population from Living Gene Bank (Jagniątków), which seemed to be distinct from the other plants from localities at high altitudes in the Karkonosze. In the Beskidy, the two branches located in one cluster represented plants from two localities: Hala Miziowa and Hala Cebulowa.

Discussion

The analysis of *A. sibiricum* specimens showed a high morphological and genetic differentiation and a low intrapopulation differentiation among the groups of the studied populations. The variability in plant populations collected in the Karkonosze

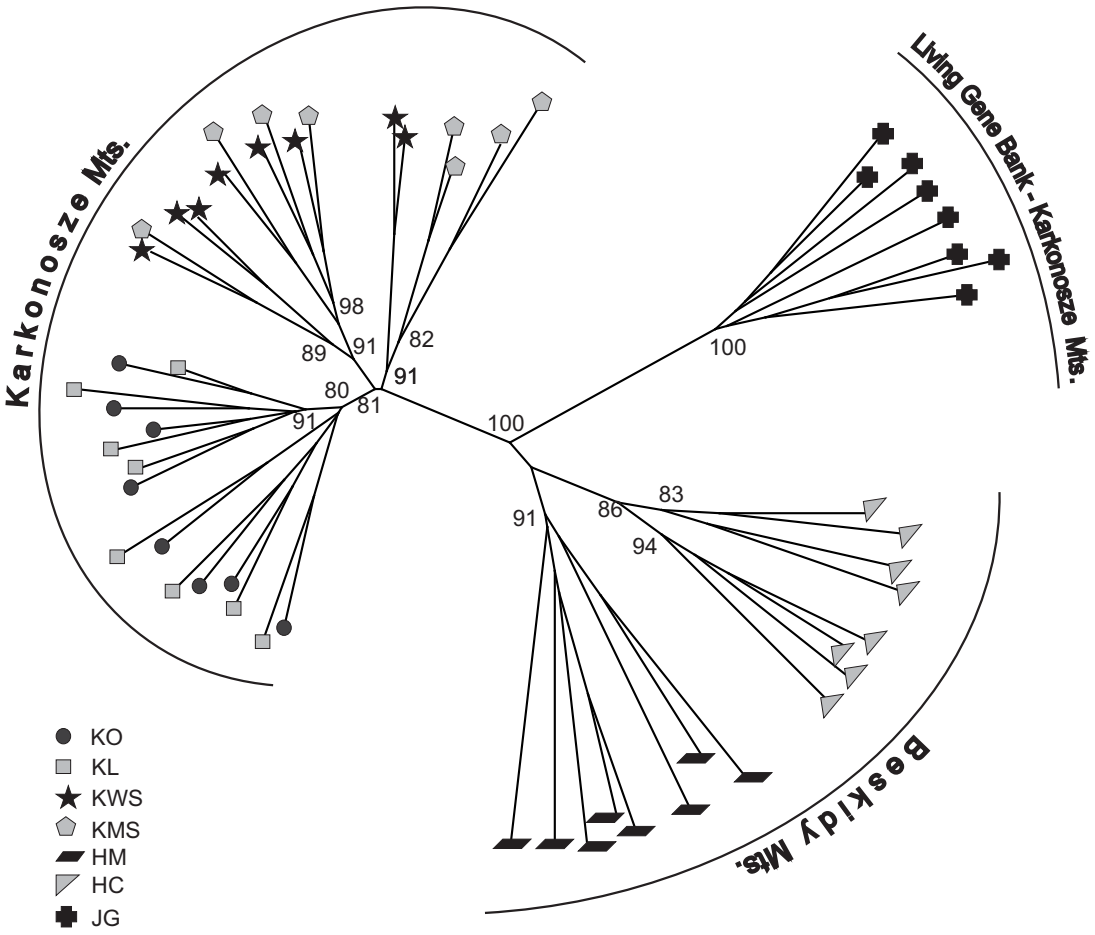

Fig. 4 Unrooted neighbor-net of *Allium sibiricum* L. individuals based on Nei and Li [40] coefficient calculated from ISSR data. Population abbreviations are the same as in Tab. 1.

and Beskidy seems to reflect the differences in the appearance of the plants which can easily be observed in both disjunctive regions [10,11]. The results seems to confirm Friesen's [11] opinion that *A. sibiricum* is a very morphologically diverse species and that the classification into three morphotypes informally described by Stearn [44] is insufficient. The typical *A. sibiricum* (*A. schoenoprasum* morphotype C) which fits the original description given by Friesen [11] was only found in the Karkonosze Mts in Poland (except for the plants from Living Gene Bank, that probably come from unknown wild origin). It grows in places which are hard to reach and are located far from tourist patches such as in glacial cirques, on wet slopes belonging to the native flora of the Karkonosze. The specimens from the Beskidy definitely did not fit to this description of *A. sibiricum* (syn. *A. schoenoprasum* morphotype C).

In fact, all of the studied populations in the Karkonosze and Beskidy exist on relatively small areas, and the genetic variability between the geographical groups of populations was high, and the morphological and molecular differentiation observed cannot easily be explained by the processes of climate oscillations in the Quaternary, as is the case with different mountain species [45,46]. On the other hand, the intrapopulation homogeneity of the populations and the high degree of interpopulation genetic divergence between the regions (Karkonosze and Beskidy mountains) can suggest isolation due to distance and a lack of gene flow between them.

Both geographical regions where *A. sibiricum* occurs are disjunctively separated in Poland and no identified hybridization areas exist between them (Sudetes and West Carpathians). While gene flow could affect the increasing level of genetic variation of the *Allium* populations, it probably does not occur because of the long geographical distance. This process is more probable between the populations in the Karkonosze Mts, where plants are distributed over a smaller area and pollinators can neutralize the negative effect of genetic drift, which can lead to a reduced genetic variation [47,48].

The results of STRUCTURE analysis indicated that some specimens from the *Allium* populations contained mixed or different genotypes. This was not only observed in the plants collected on natural sites or in the subalpine zone, but, in particular, in plants from the Living Gene Bank (Jagniątków) (Fig. 3). One of the reasons for such findings could be that these plants were probably mixed in the past: plants from natural subalpine sites, "wild" with diversely "cultivated" *Allium* sp. plants, and results in a separate genotype of plants nowadays. It is also possible that plants from the Living Gene Bank are from botanical garden seed exchange and unknown wild origin. The studied specimens from the Living Gene Bank were also morphologically different from the plants collected in high mountain localities in the Karkonosze (Tab. 2), which was confirmed by the statistical tests. On the other hand, hybridization in *Allium* plants is probably more frequent and may occur in other populations located in the Karkonosze. In September 2017, one of the authors (JU) repeatedly observed small areas of the garlic on the northern slope of Śnieżka Mt and in the vicinity of Czarny Grzbiet. This may indicate that the reproductive biology of *Allium* plant populations is dependent on several factors, not only natural processes, but most probably increasing anthropopressure, which can affect genetic variability.

Another important fact closely associated with the ease of reproduction of the genus *Allium* is its economic importance for humans. It is possible that some cultivated plants were accidentally brought along with more recent migrations. Although there is no confirmed hypothesis about the detailed origin of the *Allium* species in the Sudetes and Carpathians, it seems that *Allium* is a species which was probably broadly distributed in Europe during the glacial period [49]. The only localities where these plants exist in their natural habitat in Poland are in the Karkonosze Mts, belonging to the *A. schoenoprasum* morphotype C [11]. All studied populations occur in hard to reach places at the alpine level above 1,000 m a.s.l., in humid localities, along streams, on rocks and stony ground, and between rich vegetation close to glacial cirques (Łomniczka, Wielki Staw).

The plants growing in the Pilsko massive (Beskidy Mts) today appear to have a more complicated history. It is possible that populations of *A. sibiricum* were present on scattered sites in Pilsko such as marshes and tall herbs in the vicinity of coniferous forests, which were the natural habitats of *A. sibiricum* in the past. Later, the species probably spread due to the formation of shepherd's pastures, i.e., extensive open and wet places; hence, the current populations of the species comprise thousands of specimens. This seems to be the phenomenon of enlarging the local range of a natural species due to specific forms of anthropopressure, and could be one reason why both populations in Pilsko (Hala Miziowa and Hala Cebulowa) are rich in plants today – more than a thousand plants grow there. *Alium sibiricum*, probably hybridized with the cultivated plants introduced by Vlach shepherds –while grazing their sheep (in southern Poland in the sixteenth–seventeenth centuries and used *Allium* sp. as a seasoning plant) [50]. This is why these plants are morphologically similar to the cultivated *Allium* species but genetically differ from the natural populations in the Karkonosze. Both locations in the Beskidy, Hala Cebulowa and Hala Mizowa, were used as pastures in the past. This means that the populations from Pilsko may have a partial anthropogenic character (easy accessibility to the area, a peat bog complex surrounded by grazing areas). Similarly, large and numerous populations were observed by one of the authors (PK) in Oravska Magura and Velka Fatra, which are mountain ranges located in Slovakia, where the contemporary geographical distribution of *Allium* plants might have been created in a similar manner as those that grow in the Pilsko massive.

In conclusion, our results do not allow for clear taxonomical separation of plant specimens growing in two distinct geographical regions (Karkonosze and Beskidy). Plants from the Karkonosze definitely belong to the previously described typical *A. sibiricum* (*A. schoenoprasum* morphotype C) [11]. However, *A. sibiricum* is a very diverse species and with numerous morphologically different plants specimens. Similar plants, such as those in the Beskidy, can also be found in numerous other localities, and especially in places where human activity was intense in the past or where there is presently strong anthropogenic pressure. Plants from Hala Miziowa and Hala Cebulowa are definitely different from other plants in the Polish or Czech Karkonosze, probably due to the long-lasting anthropogenic pressure in the Carpathians. Nevertheless, it is possible that plants from the Beskidy taxonomically belong to the morphologicaly variable *A. sibiricum* (*A. schoenoprasum* morphotype C).

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